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Comparison of Several Physiological Monitoring Techniques as Applied to the Blue Mussel 'Mytilus edulis' Along a Gradient of Pollutant Stress in Narragansett Bay, Rhode Island

(U.S.) Environmental Research Lab.
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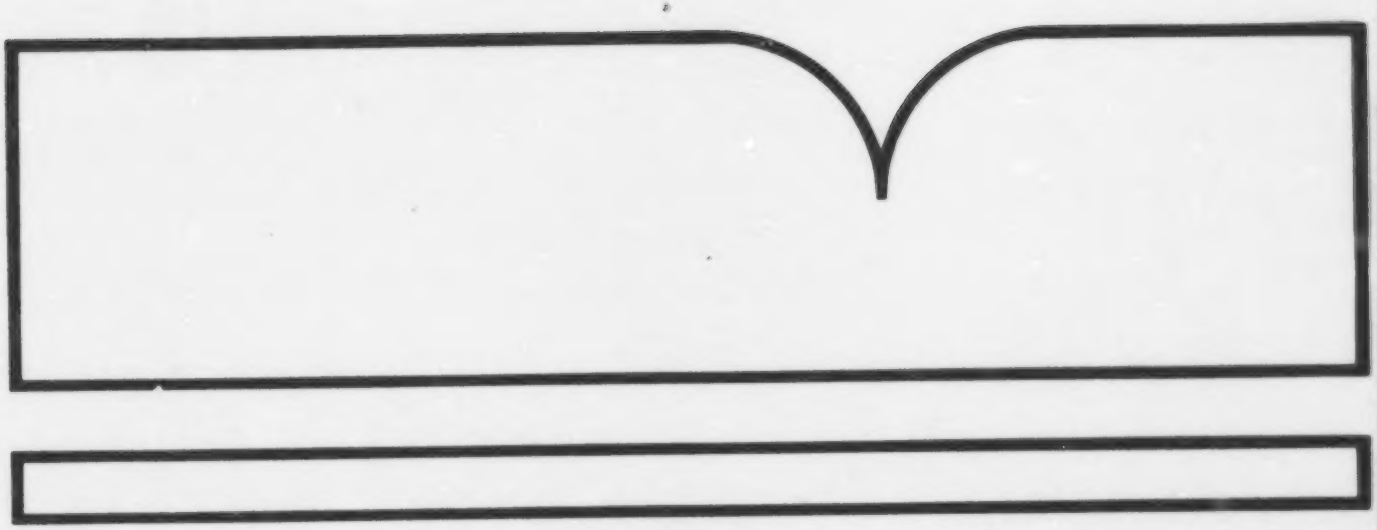

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16. ABSTRACT

Sublethal pollutant stress in mussels was clearly detected in a field-monitoring situation and provided the following evaluation of several criteria of physiological response: (1) Scope for growth and gill tissue oxygen consumption are both valuable indices of metabolic stress in mussels. (2) Examination of enzyme activity in mussel gill and posterior adductor muscle confirmed the results of gill tissue oxygen consumption and the scope for growth. (3) Hemolymph ion and adenylate energy charge measurements did not detect pollutant stress in mussels held at the polluted field station of this study. (4) The relative abundance of pinnotherid crab commensals we consider to be an interesting potential indicator of relative pollution in the field. (5) Analysis of animal tissues for specific toxicants has been demonstrated to be a reliable indicator of relative degree of pollution in the field. In the present study, both nickel and petroleum hydrocarbons reflected the stress gradient in Narragansett Bay. Relative levels of contaminants in tissues alone, however, are very difficult to interpret and have limited application. This study preents the necessary coupling of tissue residue analyses to biological effects, a blending of chemistry and biology that is a powerful tool fro field monitoring.

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Book Chapter

COMPARISON OF SEVERAL PHYSIOLOGICAL MONITORING TECHNIQUES AS APPLIED TO THE BLUE MUSSEL, *MYTILUS EDULIS*, ALONG A GRADIENT OF POLLUTANT STRESS IN NARRAGANSETT BAY, RHODE ISLAND

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INTRODUCTION

Narragansett Bay, Rhode Island, has characteristics that make it attractive as a field laboratory. At any one point in time the physical characteristics of its bottom waters are relatively constant from its mouth to its head: temperature, for example, varies little more than 2-3°C, although on an annual basis it may undergo an excursion from -1° to 26°C, essentially from arctic to tropical conditions. Salinity remains relatively constant throughout the year, within a 4°/‥ range.

The industrial history of the Bay area is a particularly useful factor in field-monitoring work. The first large-scale industry established in the United States was the Slater Mill, located on the Blackstone River, a major

tributary to the Bay (Phelps and Galloway, 1980). Pollutant input has increased since the time of that initial textile effluent in 1793, from a variety of industries and from a heavily populated metropolitan area comprising several cities. Man's activities have thus produced a pollution gradient in the Bay that overrides physical variation as a controlling factor in the distribution of resident marine species, especially in the upper reaches of the Providence River. Elevated levels of metals and some organic chemicals have been found in Bay sediments, in the water column, and in the tissues of animals taken from Bay waters (Farrington and Quinn, 1973; Phelps and Myers, 1977; Phelps and Galloway, unpublished data). Stress in marine animals exposed to even low levels of some of these pollutants has been repeatedly demonstrated, as for example petroleum hydrocarbons (Jeffries, 1972; Anderson, 1979), heavy metals (Calabrese *et al.*, 1977), and pesticides (Miller and Kinter, 1977; Christiansen and Costlow, in press).

Because the waters of this ocean estuary present a gradient of pollutant stress from its contaminated head to its relatively clean mouth, the Bay thus provides an excellent opportunity to examine, in a field laboratory, some effects on marine life of pollution arising from man's activities. Such a study was performed in 1977, using the criterion scope for growth, a multiparametric index of whole-animal respiration and feeding rates reflecting gross and net growth efficiencies (Widdows *et al.*, in press). Four stations were established on a transect along the pollution gradient in Narragansett Bay, from a highly polluted site (Station 1) to a relatively clean site (Station 4), shown in Figure 1. Blue mussels (*Mytilus edulis*) were exposed for 1 month at each of these four stations and their scope for growth measured in August and in October. Scope for growth data reflected the stress gradient in both months, and the O:N ratio alone was a good stress indicator in August, but not after spawning, in October (Widdows *et al.*, 1979). At the same time, the concentration of several heavy metals in mussel tissues was determined; data for lead, cadmium, and iron showed no particular pattern, but nickel reflected the stress gradient (Fig. 2), and analysis of the water column gave similar results, inversely proportional to scope for growth. A subsequent laboratory study with mussels, involving a 96 hr-exposure to 1000 ppb Ni failed to establish significant physiological stress, as determined by gill respiration (Thurberg, unpublished data). Nickel is strongly related, however, to the degree of overall pollution in our field stress gradient. Petroleum hydrocarbons in mussel tissues also followed the stress gradient (Fig. 3).

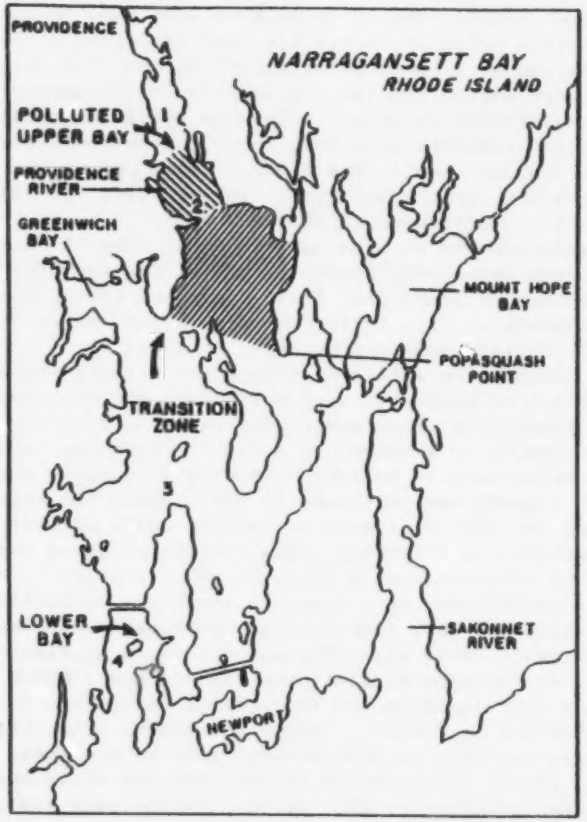

FIGURE 1. Map of Narragansett Bay showing the sites where mussels were held from April to September, 1979. Station 1 is a heavily polluted station, where the animals all died very quickly; Station 2, the next most polluted, and Station 4, a relatively clean area near the mouth of the Bay, were used for the field exposures in this study.

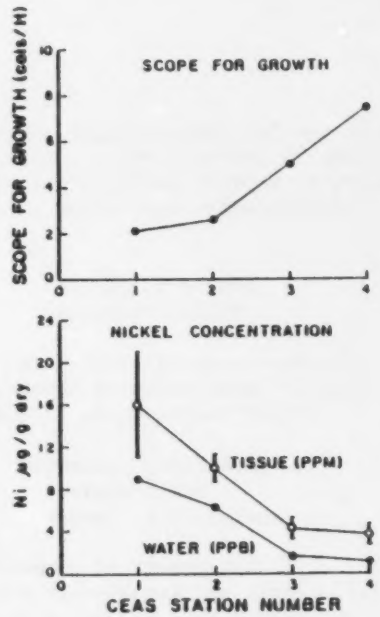

FIGURE 2. Nickel concentrations in M. edulis tissues (open circles) and water column (solid circles) and scope for growth in mussels taken at the same time from 4 stations in Narragansett Bay, R. I. (from Phelps and Galloway, 1980). See Figure 1 for station sites.

At this point, it seemed desirable to obtain data for more than a single month's exposure, and to examine the relative effectiveness of other biological stress indicators. After a feasibility study in the summer of 1978, therefore, we undertook the work reported here. Because the mussels set out at the heavily polluted Station 1 did not survive through the summer, we used Station 2 as the polluted site and Station 4, the cleanest site, for comparison, setting the animals out in April, 1979, and sampling at monthly intervals from May to September.

The frequent sampling and the large number of animals involved dictated our choice of stress criteria, physiological and biochemical measurements that are relatively simple and rapid to perform: gill-tissue respiration, serum-ion concentrations, and energy-related enzyme activities in

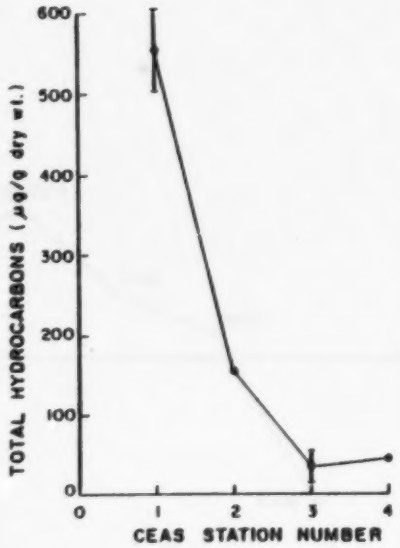

FIGURE 3. Total petroleum hydrocarbons in tissues of M. edulis held at stations in the Narragansett Bay pollution gradient (from Phelps and Galloway, 1980).

gills and posterior adductor muscle. We also report on another suggested stress criterion, the adenylate energy charge, performed in 1978 on mussels held at Stations 2 and 4.

METHODS AND MATERIALS

Mussels were distributed and held along the Bay transect in submerged plastic baskets as described earlier by Phelps and Galloway (1980).

Twenty-five mussels from Station 2 and Station 4 were brought on ice to Milford, Connecticut, and kept in running seawater (22±2 ppt; 19±2°C) for 12 to 14 hours. Twenty animals of approximately equal size from each station were then prepared for respiration, serum ion, and biochemical studies. Hemolymph was sampled from the posterior adductor muscle via syringe and 22-gauge needle, then centrifuged to remove debris. Osmolality was read on an Advanced 3L

osmometer[1], and serum sodium, potassium, and calcium were determined with a Coleman flame photometer. Gills from one side of each mussel were removed and placed in a 15-ml Warburg-type flask, one flask per mussel. Each flask contained 5 ml seawater from the holding tank. Oxygen consumption was monitored over a 4-hr period at 20°C in a Gilson Differential Respirometer. Oxygen-consumption rates were calculated as microliters of oxygen consumed per hr per g dry weight of gill tissue ($\mu l\ hr^{-1}\ g^{-1}$), corrected to microliters of dry gas at standard temperature and pressure.

For biochemical examination, pooled gill-tissue homogenates were prepared immediately after excision of the tissues, then frozen-stored at -80°C until testing; posterior adductor muscle was cleaned and packaged in air-tight plastic bags, then held at -80°C until testing. Tissues were homogenized in all-glass equipment as follows: fresh gill tissue, 1:4 w/v, in cold doubly glass-distilled water; PAM[2], 1:9, w/v, in cold 1% Triton, a non-ionic surfactant (W-1339, Ruger Chemical Company, Irvington-on-Hudson, N.Y.). Centrifugation was at 4°C and 40,000 g for both tissue homogenates, 45 min for gill (after thawing frozen preparations in RT tap water) and 60 min for PAM. The clear supernates, E^{5x} gill and E^{10x} PAM, were used for enzyme analyses.

Enzyme assays

All solutions were made in doubly glass-distilled water. Biochemicals were obtained from Sigma Chemical Company, St. Louis, Mo. Activities of all enzymes were determined by standard fixed-wavelength spectrophotometry using pyridine nucleotide coenzymes either directly or by a coupled-assay procedure; assay temperature was 25°C.

[1]*Use of trade names does not constitute endorsement by the NMFS, NOAA, or by the EPA.*

[2]*Abbreviations used: PAM, posterior adductor muscle; NADH, reduced form of nicotinamide adenine dinculeotide; NADP, nicotinamide adenine dinucleotide phosphate; EDTA, ethylenediamine tetraacetic acid, dipotassium salt, dihydrate; cis-OA, cis-oxaloacetic acid; ADP, adenosine diphosphate; PEP, phospho(enol)pyruvate; Tris, tris(hydroxymethyl)-aminomethane; G6P, D-glucose-6-phosphate, monosodium salt; AAT, aspartate aminotransferase, E.C. 2.6.1.1; G6PDH, glucose-6-phosphate dehydrogenase, E.C. 1.1.1.49; LDH, lactate dehydrogenase, E.C. 1.1.1.27; MDH, malate dehydrogenase, E.C. 1.1.1.37; PK, pyruvate kinase, E.C. 2.7.1.40.*

Unit of activity in each case was μmoles NADH oxidized or NADP reduced per min per mg biuret protein. Assay concentrations (mM) in a 3.00-ml reaction volume were: MDH=buffer pH 9.0 glycine (90) and EDTA (0.9), NADH (0.15), cis-OA (1.0), and 0.10 ml E^{100x} (1:19, v/v with water, for E^{5x} gill; 1:9 v/v with water, for E^{10x} PAM); PK=glycylgycine buffer pH 8.0 (60), NADH (0.15), $MgCl_2$ (8.3), KCl (7.5) ADP (0.31), PEP (0.53), 175 units LDH, and 0.10 ml E^{5x} gill or E^{10x} PAM; G6PDH=Tris buffer pH 8.0 (82), NADP (0.3), $MgCl_2$ (2.5), G6P (1.0), and 0.10 ml E^{5x} gill; LDH=glycylglycine buffer pH 7.5 (70), NADH (0.15), Na pyruvate (3.3), and 0.30 ml E^{10x} PAM; AAT=K phosphate buffer pH 7.5 (90), L-aspartate, potassium salt (200), NADH (0.35), α-ketoglutarate, potassium salt (6.7), 20 units MDH, and 0.20 ml E^{10x} PAM. Determination of statistical significance was made using the Student's *t* test.

RESULTS AND DISCUSSION

Gill-Tissue Respiration

Monthly sampling of mussels held along a pollution gradient showed generally increasing oxygen-consumption rates in gills of animals taken from the polluted site, Station 2; gill-tissue respiration is a widely-used criterion of physiological stress in bivalves (Thurberg, *et al.*, 1974). Oxygen-consumption rates for animals collected at Station 2 in May were depressed as compared to Station 4 animals, but by June these rates were about equal, and from that time on animals from Station 2 had significantly elevated ($P<0.05$) oxygen-consumption values (Fig. 4), although in September at a somewhat lesser level, as was also seen in the biochemical data. These elevated rates indicate increased metabolic activity that is probably the result of the high pollution levels found at Station 2. The depressed rates of oxygen consumption in May during the early period of exposure to Station 2 waters may have been due to decreased activity, shell closure, and anerobic metabolism, all in an initial effort to "close out" unfavorable environmental conditions.

These elevated summer gill-respiration rates corroborate the results of the earlier scope for growth study (Widdows *et al.*, in press). In that study, mussels were held at stations along the Narragansett Bay pollution gradient for one month before testing. In both August and October tests, scope for growth was significantly depressed at the polluted sites (Fig. 2). This means that an abnormally high amount of available energy was used for maintenance and little was left

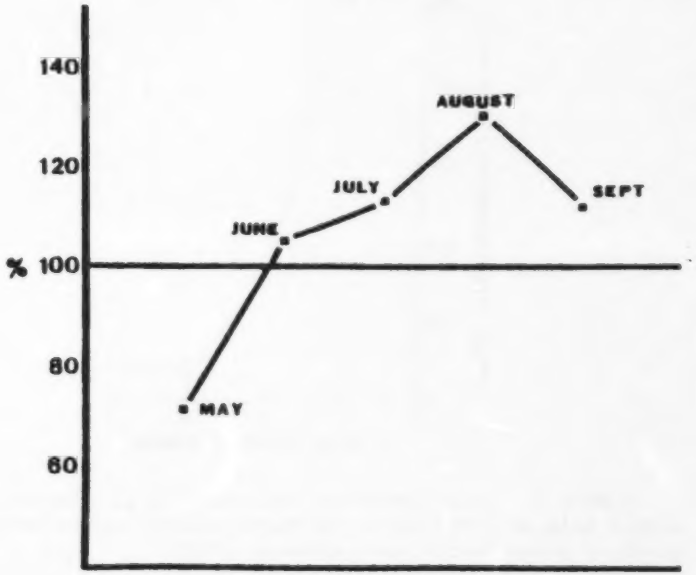

FIGURE 4. Gill-tissue oxygen consumption of M. edulis sampled monthly at two stations in Narragansett Bay, R.I. The horizonal line in the middle of the figure represents data for animals from the relatively clean Station 4, taken as unity, and the points connected by lines represent data for animals taken from the polluted Station 2, presented as percent of values for the clean station.

over for growth. In the present study, the high gill-tissue oxygen-consumption measurements for animals taken July-September at the pollutant site indicate the same condition in mussels held along the gradient for the entire summer season.

Both of these physiological indices of metabolic stress provide similar information, and both have advantages and disadvantages that must be weighed before selection for a monitoring program. Scope for growth gives a much more complete metabolic picture, including feeding rates, oxygen-consumption rates, and oxygen:nitrogen ratios - all incorporated in a single index (Bayne *et al.*, 1980). A disadvantage of this measure is the requirement for a time-consuming suite

of measurements and a considerable variety of equipment, which limits the number of animals that can be sampled and measured for any given point in time. On the other hand, gill-tissue oxygen consumption, although it gives only one part of the metabolic picture, can be performed on many animals in a short period of time, and can also be easily measured in a shipboard laboratory. For many studies this simple indicator of metabolic activity is sufficient, especially where numerous stations and many animals must be sampled to obtain a statistically significant sample size. Such a large sample size is often necessary to filter out the effects of the many environmental factors that influence metabolic activity (Thurberg, 1980).

Gill Enzyme Activity

Monthly sampling of *Mytilus* gills for enzyme analysis during the 1979 field study showed that glycolytic activity (as measured by PK) in control mussels at Station 4 dipped from a high in April and May, when the animals first spawn, to a low in June and July and then rose again, reaching the levels of late April by early September (Fig. 5). Bayne and Thompson (1970) observed that the general tissue metabolism of *Mytilus* is depressed in early summer, after spawning, and both gill glycolysis and gill respiration in this field study agree with their findings. In animals from the polluted Station 2, gill glycolysis followed a similar course but at significantly lower levels, both at the end of spawning in early May ($P<0.01$) and June ($P<0.005$) and during renewed glycolytic activity in early September ($P<0.01$). The markedly lower gill glycolysis in polluted mussels in May, and the diminished capacity in September to sustain the summer's glycolytic surge, both confirm the gill respiratory data.

Despite wide sample variability in May and June, biosynthetic activity in the gill (as measured by the pentose shunt enzyme G6PDH) was observed to rise steadily in clean *Mytilus* from April to September (Table 1). In animals from the polluted station, however, data indicate somewhat lower pentose shunt activity in June, possibly the result of an abnormally high ammonia concentration in the water at that station at that time (Table 2), the only hydrographic parameter being monitored that was markedly different from values at the clean station.

We favor this explanation in the light of the following observations: In August of the previous year, ammonia values at the polluted station rose to 18 and 20 μmoles l^{-1}, accompanied by a decrease in dissolved oxygen levels.

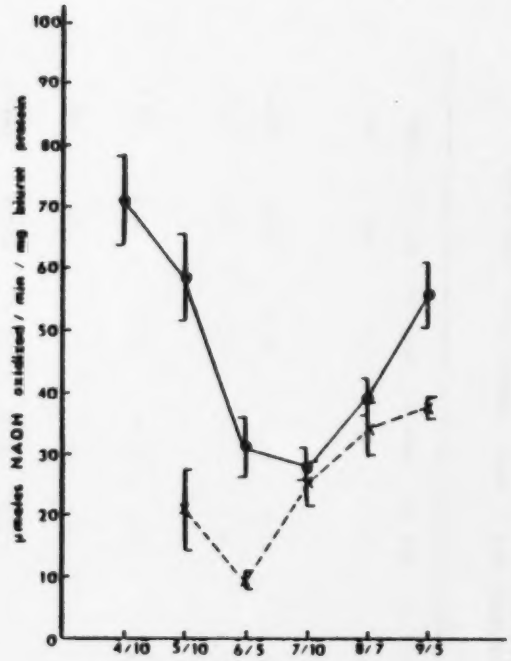

FIGURE 5. Pyruvate kinase activity in gill tissue of M. edulis *sampled monthly at No. 2, polluted (x----x), and at No. 4, clean (o----o), stations in Narragansett Bay, R.I. Each point represents the arithmetic mean for 6-9 sample pools, 2-3 animals per pool, and the bars represent standard error. Differences between the two stations were highly significant in May, June, and September.*

TABLE 2. Hydrographic parameters at Station 2 (polluted) and 4 (clean) in Narragansett Bay during the summer of 1979.

		June 4	July 9	Aug 6	Sept 4
Sta. Temp.: (°C)	Sta. 2	14.4	19.5	22.2	21.8
	4	13.8	18.6	22.6	21.8
Sta. Sal.: (o/oo)	Sta. 2	26.5	26.9	30	27.4
	4	29.5	30.6	28	30.2
D.O.: (mg l^{-1})	Sta. 2	5.08	6.01	1.49	4.25
	4	8.16	8.04	5.82	6.55
NH_3: (µmoles l^{-1})	Sta. 2	15.65	1.27	----	8.50
	4	1.12	2.35	----	2.50

Although for our preliminary work we sampled on only 3 dates that year (July, August, October), we found that mussels taken in August from that same polluted station showed significant stress, both in elevated gill-tissue respiration ($P<0.01$) and in elevated activity of adductor muscle NAT ($P<0.001$) and gill-tissue MDH ($P<0.005$), both enzymes that have served as criteria of sublethal metal stress in tissues of marine crustaceans (Gould *et al.*, 1976; Gould, 1980). Because of the possible implication of high ammonia levels in the 1978 observations of stress in mussels at the polluted site, therefore, we exposed healthy *Mytilus* (from the population set out in the field in April of 1979) for 22 days to 20 µmoles NH_3 l^{-1} (as NH_4Cl) in basins of aerated water that was changed daily, and sampled control and exposed animals at 1, 8, 15, and 22 days. G6PDH activity was lower in the gills of exposed mussels at 22 days ($P<0.05$), an indication of lower biosynthetic rates, and the same animals had depressed hemolymph potassium ($P<0.05$). On the other hand, gill respiratory rates for these animals did not differ from the controls; we conclude, therefore, that a high ammonia level alone is not sufficient to produce the physiological stress signals observed in August 1978. But the lower gill G6PDH activity in the ammonia-exposed mussels of this brief laboratory study, together with this year's field observation linking decreased gill G6PDH and high ammonia concentrations, suggest that further work in this area could prove fruitful.

Gill MDH activity was not significantly different in the polluted animals during the 1979 field study.

TABLE 1. G6PDH Activity (µmoles NADP reduced min^{-1}) in gills of *Mytilus edulis* taken at monthly intervals in 1979 from clean or polluted stations in Narragansett Bay. Each sample pool comprised either 2 or 3 animals.

	April 10	May 10	June 5	July 10	Aug. 7	Sept. 5
Station 4 (clean) (N) pooled samples	(8)	(6)	(6)	(9)	(9)	(8)
$\bar{x}$	37.5	61.9	85.2	91.9	95.1	106.1
S.E.	1.5	18.7	21.8	13.3	9.6	13.5
% sample variability, 100 (S.E./$\bar{x}$)	4.0	30.2	27.9	14.5	10.1	12.7
Station 2 (polluted) (N) pooled samples	(6)	(6)	(8)	(6)	(8)	
$\bar{x}$	73.2	46.3	122.3	117.7	108.3	
S.E	21.7	11.6	18.0	12.9	5.2	
% sample variability, 100 (S.E./$\bar{x}$)	29.6	29.4	14.7	11.0	4.8	

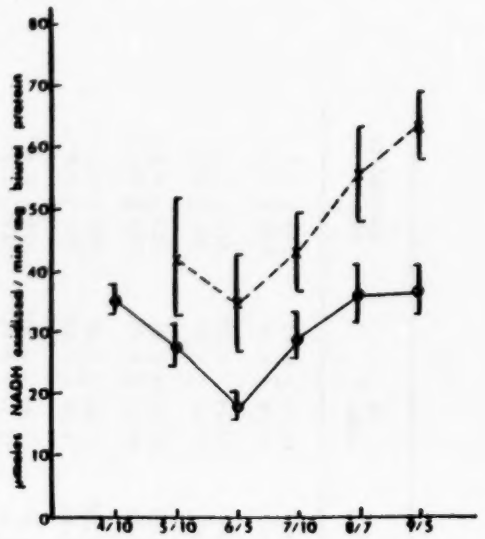

FIGURE 6. Lactate dehydrogenase activity in posterior adductor muscle of M. edulis *sampled monthly at No. 2, polluted (x----x), and No. 4, clean (o----o), stations in Narragansett Bay, R.I. Each point represents the arithmetic mean for 8-15 samples, and the bars represent standard error. Differences between the stations were highly significant in August and September.*

Adductor Muscle Enzyme Activity

In the posterior adductor muscle, the only statistically significant change observed was an increasingly elevated LDH activity (Fig. 6) in animals from the polluted station in August ($P<0.05$) and September ($P<0.005$), a possible indication that the sublethally stressed animals were drawing upon energy reserves in the muscle to support a growing metabolic demand in the increasingly stressed gill, the latter evinced by the weakening PK in September (Fig. 5). This interpretation would support the observations of Dunning and Major (1974), who, working with *Mytilus* exposed to sublethal levels of oil, found glycogen stores of the posterior adductor muscle to be depleted, although normally this animal retains muscle glycogen for use during gametogenesis, and will not draw upon this energy reserve even during prolonged starvation.

When gill PK and G6PDH and adductor muscle LDH in the polluted animals are calculated as percent of values for animals from the clean stations, two noteworthy patterns emerge (Fig. 7). First, the gill tissue enzymes follow the same course as do the similarly plotted gill oxygen-consumption data (Fig. 4): an even greater depression in the polluted animals than in control animals after spring spawning, followed by an overcompensation that gradually lessens. The second pattern is the picture of inversely proportional activity of adductor muscle LDH, in comparison with the gill tissue enzymes; in the pollutant-stressed animals, energy reserves of the posterior adductor muscle are apparently mobilized to support the weakened gill metabolism. The "sparing" of adductor muscle glycogen stored for gametogenesis, therefore, does not operate effectively in pollutant-stressed mussels.

Hemolymph Ions

Although some differences in hemolymph ion concentration were observed (Table 3), they did not reflect the pollutant stress gradient indicated by the other measures of physiological condition tested. Mussels are reported to have no osmoregulatory ability, very little ion-regulation, and a slow adaptation to any change of salinity (Shumway, 1977). In this study, the mussels were taken from two stations of similar salinities (Table 2), then placed in a significantly lower salinity overnight before testing (see Methods and Materials). For any future work of a similar nature, it may be advisable to bleed the mussels immediately after their removal from the stations in the pollution gradient. In the light of our results here, however, hemolymph ion concentrations did not reflect field stress.

Adenylate Energy Charge

The AEC data for mussels field-collected at Stations 2 and 4 did not support the picture of stressful field conditions indicated by other physiological criteria. The data did appear to reflect a seasonal change in condition of all field-exposed animals between April and August, the two sampling dates. The ATP: AMP ratio seemed more promising as a stress indicator, but the sample number was insufficient to prove a statistically significant difference between the two groups. From the results of this attempt to apply this energy-intensive and time-consuming technique to field studies, we do not consider the AEC a practical monitoring tool.

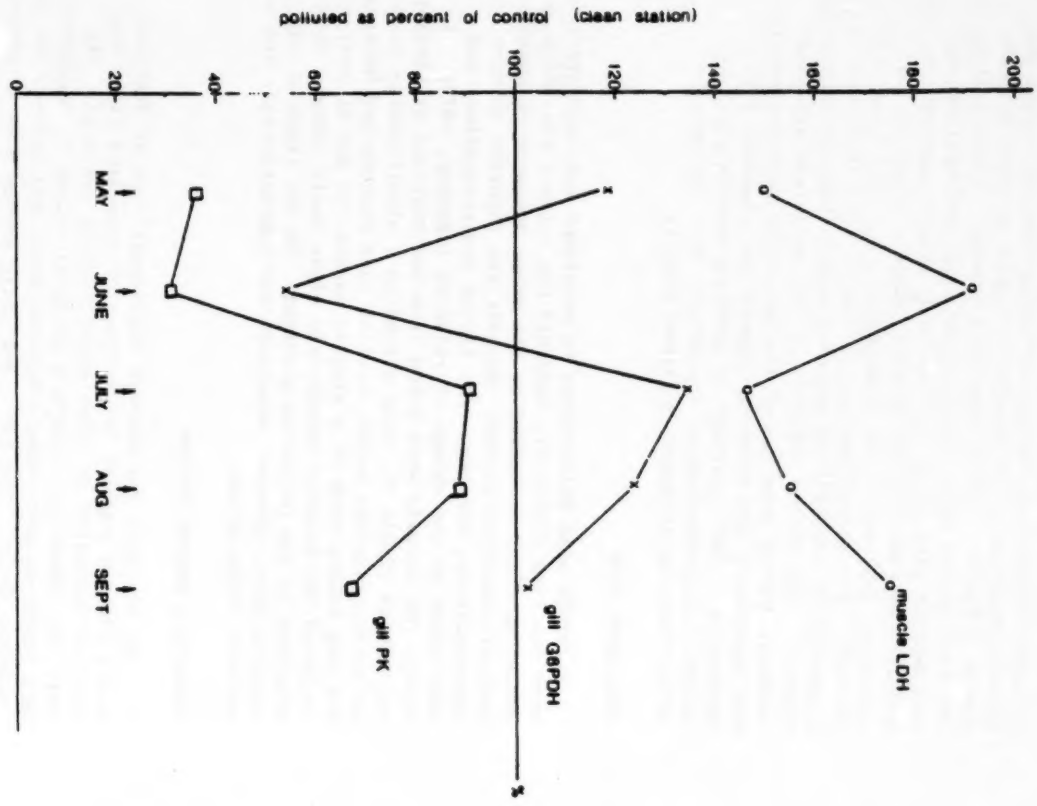

FIGURE 7. Posterior adductor muscle LDH and gill PK and G6PDH activities in M. edulis from polluted Station 2, as percent of corresponding activities in animals from clean Station 4.

TABLE 3. Hemolymph ion concentration and osmolatities of mussels taken from station 2 (polluted) and Station 4 (clean) during the summer of 1979.

		Na $mEq\ l^{-1}$	K $mEq\ l^{-1}$	Ca $mEq\ l^{-1}$	Osmolality mOsm
May	St. 2	248 ± 2.6	9.53 ± 0.51	10.57 ± 0.81	723 ± 1.7
	St. 4	253 ± 2.5	9.09 ± 0.63	9.76 ± 0.2[illegible]	735 ± 5.9
June	St. 2	313 ± 4.6	10.33 ± 0.64	12.54 ± 0.28[a]	827 ± 14.5
	St. 4	379 ± 3.7[a]	11.25 ± 0.60	17.11 ± 0.58	861 ± 10.0
July	St. 2	344 ± 3.5	10.17 ± 0.40	16.05 ± 0.32	763 ± 4.4
	St. 4	335 ± 2.2	11.10 ± 0.71	16.17 ± 0.29	765 ± 2.0
August	St. 2	309 ± 1.6[a]	11.43 ± 0.50[a]	15.86 ± 0.29	792 ± 4.1
	St. 4	353 ± 5.0	9.48 ± 0.25	16.06 ± 0.53	796 ± 3.9
September	St. 2	313 ± 3.9[a]	9.03 ± 0.27	12.52 ± 0.30[a]	775 ± 0.5[a]
	St. 4	293 ± 2.3	9.91 ± 0.51	11.31 ± 0.25	764 ± 1.2

All values are mean of 15-20 samples ± standard error.

[a]*Significantly different from St. 4; P < .05.*

Pea Crabs

We also took note of the pinnotherid crabs (*Pinnotheres maculatus*) living as commensals within the mantle cavity of the mussels collected from Stations 2 and 4. In June, half of the 20 mussels examined at each station contained a single crab. During July, August, and September, however, mussels from the polluted Station 2 contained only one-third to one-half as many crabs as mussels from the cleanest station (Table 4). These limited data suggest that the crabs either died at the polluted station or left the mussels in an attempt to escape unfavorable environmental conditions. They also support the theory that a lack of pinnotherid commensals in certain areas may be directly related to the level of pollution (J. B. Pearce, pers. comm.).

Tissue Residue Analysis

Another monitoring tool is the chemical analysis of body tissues for specific pollutants (Goldberg *et al.*, 1978). Such analyses were performed on *Mytilus* tissues collected during the summers of 1977 through 1979. Significant findings were that of the metals tested, nickel concentrations reflected the pollution gradient in the Bay (Fig. 2), as did petroleum hydrocarbons (Fig. 3), and that the metal levels remained remarkably stable over this three-year period of observation (Phelps and Galloway, unpublished data).

TABLE 4. Pea crabs (*Pinnotheres maculatus*) found within the mantle cavity of mussels collected from Stations 2 (polluted) and 4 (clean).

	Number found at Sta. 2	Number found at Sta. 4	Sta. 2 as % Sta. 4
June 4	10	10	100
July 9	7	14	50
Aug. 6	2	6	33
Sept. 4	5	10	50

SUMMARY

Sublethal pollutant stress in mussels was clearly detected in a field-monitoring situation using these criteria of physiological response: scope for growth (Widdows *et al.*, 1980), tissue residue analysis (Phelps and Galloway, 1980), and gill respiration and glycolytic rates in either gill or posterior adductor muscle (this report). We have drawn the following assessment of their relative usefulness in this field study of pollutant stress:

1. Scope for growth and gill-tissue oxygen consumption are both valuable indices of metabolic stress in mussels. Scope for growth is the more comprehensive index and the more labor-intensive. It has the advantage of relating an index of condition directly to such vital whole-body functions as growth and reproduction. Gill respiration is a simpler and faster measure that can be used with larger numbers of animals in a short period of time with limited laboratory space available, as at sea. It does not, however, provide direct information on feeding rates or nitrogen metabolism.

2. Examination of enzyme activity in mussel gill and posterior adductor muscle confirmed the results of gill-tissue oxygen consumption and the scope for growth. Such measurements of subcellular metabolic activity also provide information on the flow of and capacity for energy expenditure, and often give earlier and more sensitive indication of metabolic stress, picking up significant differences before whole-tissue or whole-animal response is detectable.

3. Hemolymph ion and adenylate energy charge measurements did not detect pollutant stress in mussels held at the polluted field station of this study. We therefore conclude that these physiological indices are not appropriate for monitoring biological response to pollutant stress in this animal, at least, under field conditions.

4. The relative abundance of pinnotherid crab commensals we consider to be an interesting potential indicator of relative pollution in the field.

5. Analysis of animal tissues for specific toxicants has been demonstrated to be a reliable indicator of relative degree of pollution in the field (Goldberg *et al.*, 1978; Phelps and Galloway, 1980). Levels of nickel in *Mytilus* tissues for each of the four stations were remarkably stable over a three-year testing period, and both nickel and petroleum hydrocarbons reflected the stress gradient in Narragansett Bay. Relative levels of contaminants in tissues alone, however, are very difficult to interpret and have limited

application. This study presents the necessary coupling of tissue residue analyses to biological effects, a blending of chemistry and biology that is a powerful tool for field monitoring.

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